IC Application Note P–50

Determination of steviol glycosides in sweetener applying flexIPAD detection



Stevia is a plant of the Compositae family whose leaves can be used as sweetener. For usage in the food sector, the steviol glycosides in stevia need to be controlled for purity. The use of the flexIPAD waveform results in higher response than standard PAD. Separation is performed on a RP column. Post-column addition of sodium hydroxide enables amperometric detection.

Results

| Compound | Concentration injected [mg/L] | Concentration in product [mg/kg] |
|----------------|----------------------------------|-------------------------------------|
| Rebaudioside A | 414 | 41 |
| Stevioside | n.q. | n.q. |
| Rebaudioside C | n.q. | n.q. |



Sample

Stevia sweetener, powder

Sample preparation

Samples are dissolved in eluent and directly injected.

Columns

| Luna 5 µ (Phenome | m C18, 2 enex) | 250 × 4.6 | mm | 00G-4252-E0 |
|----------------------|-------------------|-----------|-----|-------------|
| Security (Phenome | Guard enex) | Cartrige | Kit | KJO - 4282 |

Solutions

| Eluent | 10 mmol/L sodium dihydrogen phosphate, pH = 4.5 (phosphoric acid); acetonitrile 70:30 (v/v) |
|----------------------|--|
| Post-column addition | 400 mmol/L sodium hydroxide |

Parameters

| Flow rate | 1.0 mL/min |
|--------------------|------------|
| Flow rate (PCA) | 0.3 mL/min |
| Injection volume | 20 µL |
| Recording time | 22 min |
| Column temperature | 40 °C |

PAD Parameters

| Cell | Wall-Jet cell |
|---------------------|---------------|
| Working electrode | Gold (2 mm) |
| Reference electrode | Palladium |
| Spacer | 50 µm |
| Meas. range | 2 mA |
| Meas. duration | 420 ms |
| Cycle duration | 900 ms |
| Temperature | 35 °C |
| Mode | flexIPAD |
| Measuring mode | Current |

Analysis

Pulsed amperometric detection

Instrumentation

| 940 Profesional IC Vario ONE/HPG | 2.940.1140 |
|--|------------|
| IC Amperometric Detector | 2.850.9110 |
| 858 Professional Sample Processor | 2.858.0020 |
| IC equipment Wall-Jet cell: without electrodes | 6.5337.000 |
| Au working electrode, 2 mm | 6.1257.260 |
| Pd reference electrode | 6.1257.740 |





flexIPAD profile

| Duration [ms] | Start potential [V] | End potential [V] |
|---------------|------------------------|----------------------|
| 370 | 0.0 | 0.0 |
| 150 | 0.0 | 0.28 |
| 110 | 0.28 | 0.28 |
| 120 | 0.28 | 0.0 |
| 50 | 0.0 | 0.0 |
| 40 | -1.0 | -1.0 |
| 60 | 0.6 | 0.6 |



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